Exhibit D

Disorders With Type III Hypersensitivity Reactions

The Merck Manual of Diagnosis and Therapy
Section 12. Immunology; Allergic Disorders
Chapter 148. Hypersensitivity Disorders
Topics

[General]
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navigation help

Conditions in which immune complexes (ICs) appear to play some role are serum sickness due to serum, drugs, or viral hepatitis antigen; SLE; RA; polyarteritis; cryoglobulinemia; hypersensitivity pneumonitis; bronchopulmonary aspergillosis; acute glomerulonephritis; chronic membranoproliferative glomerulonephritis; and associated renal disease (see Ch. 231). In bronchopulmonary aspergillosis, drug- or serum-induced serum sickness, and some forms of renal disease, an IgE-mediated reaction is thought to precede the type III reaction.

The standard animal models of type III reactions are the local Arthus reaction and experimental serum sickness. In the **Arthus reaction** (typically a local skin reaction), animals are first hyperimmunized to induce large amounts of circulating IgG antibodies an then are given a small amount of antigen intradermally. The antigen precipitates with the excess IgG and activates complement, so that a highly inflammatory, edematous, painful local lesion rapidly appears (by 4 to 6 h) and may progress to a sterile abscess containing many polymorphonuclear cells, and then to tissue necrosis. A necrotizing vasculitis with occluded arteriolar lumina can be seen microscopically. No lag time precedes the reaction because antibody is present already.

In **experimental serum sickness,** a large amount of antigen is injected into a nonimmunized animal. After a lag time, antibody is produced; when the antibody reaches a critical level (10 to 14 days in humans), antigen-antibody complexes form and are deposited in endothelial vessels, where they produce widespread vascular injury characterized by the presence of polymorphonuclear leukocytes. When vasculitis occurs, a fall in serum complement can be detected, and antigen, antibody, and complement can be found in the areas of vasculitis. The antigen-antibody complexes, however, cannot induce injury by themselves, but rather require the presence of increased vascular permeability, such as occurs in IgE-mediated (type I) reactions and when complement is activated to enhance vascular deposition of the IC.

Diagnosis

Type III reactions can be suspected in human disease when vasculitis occurs. In polyarteritis, the presence of vasculitis is the only clinical evidence to support a role for ICs. Further support may be obtained by direct immunofluorescence tests (as described above), which may indicate the presence of antigen, immunoglobulin (Ig), and complemen in the area of vasculitis.

In experimental studies, fluorescence microscopy shows a coarse granular deposit (lumpy bumps) along the basement membrane when animal glomeruli are stained for Ig and complement. A similar distribution can be seen in type III human renal diseases (see Ch. 231). The electron microscope also can be used to detect electron-dense deposits (similar tc those seen in experimental serum sickness), which are believed to be the antigen-antibody complexes. Rarely, both antigen and antibody can be detected by immunofluorescence in the inflamed tissue--this has been shown in the renal disease of SLE and in the vasculitic lesions of hepatitis-antigen-associated serum sickness.

A type III reaction is further evidenced by demonstrating the presence of circulating antibody to antigen, such as horse serum, hepatitis antigen, DNA, altered IgG (rheumatoid factor), and some molds. In SLE, for example, a rise in antibody to native undenatured, double-stranded DNA and a fall in serum complement occur during exacerbations of renal disease. If the antigen is unknown, levels of total serum complement and of the early components (C1, C4, or C2) can be tested; a depressed level indicates classic complement activation and, therefore, a type III reaction.

In allergic pulmonary aspergillosis, an intradermal skin test with *Aspergillus* antigen may produce an IgE-mediated wheal and flare reaction followed by an Arthus-like reaction.

Until recently, ICs were detected in serum by cryoprecipitation (using the property of some complexes to precipitate in the cold). Sophisticated equipment also could detect soluble complexes by analytic ultracentrifugation and sucrose density gradient centrifugation. Currently, several tests detecting circulating ICs are used based on the ability of complexes to react with complement components (eg, C1q-binding assays) and the ability of complexes to inhibit the reaction between monoclonal rheumatoid factor and IgG. Assays such as the Raji cell assay are based on the interaction of ICs containing complement components with cellular receptors (eg, a C3 receptor on the Raji cell). Although others are available, such assays are used most commonly. No single test detects all ICs, and their use in clinical medicine is limited to monitoring the activity of certain diseases.

AUTOIMMUNE DISORDERS

Disorders in which the immune system produces autoantibodies to an endogenous antigen, with consequent injury to tissues.

Considered here are the pathogenetic immunologic mechanisms underlying autoimmune diseases (see also Table 148-4). Clinical aspects of the specific disorders are discussed elsewhere in *The Manual.*

Development of the Autoimmune Response

Although precise details of the autoimmune response are not completely understood, the outcome of antigenic stimulation, whether it be antibody formation, activated T cells, or tolerance, seems to depend on the same factors with autoantigen as with exogenous antiger Five possible mechanisms for developing an immune response to autoantigens are recognized:

1. Hidden or sequestered antigens (eg, intracellular substances) may not be recognized as "self"; if released into the circulation they may induce an immune response. This occurs in sympathetic ophthalmia with the traumatic release of an antigen normally sequestered within the eye. Autoantibody alone may not produce disease because it cannot combine with the sequestered antigen. For example, antibodies to sperm and heart muscle antigens are blocked by the basement membrane of the seminiferous tubules and myocardial cell membrane, respectively. Immunologically active T cells may lack such restrictions and would produce injury more effectively.
2. The "self" antigens may become immunogenic because of chemical, physical, or biologic alteration. Certain chemicals couple with body proteins and render them immunogenic (as in contact dermatitis). Drugs can produce several autoimmune reactions (see Hypersensitivity to Drugs, below). Photosensitivity exemplifies physically induced autoallergy: ultraviolet light alters skin protein, to which the patient becomes allergic. Biologically altered antigens occur in New Zealand mice that develop autoallergic disease resembling SLE when persistently infected with an RNA virus known to combine with host tissues, altering them enough to induce antibody.
3. Foreign antigen may induce an immune response that cross-reacts with normal "self" antigen; eg, the cross-reaction that occurs between streptococcal M protein and human heart muscle.
4. Autoantibody production may result from a mutation in immunocompetent cells. Thi may explain the monoclonal autoantibodies seen occasionally in patients with lymphoma.
5. Autoimmune phenomena may be epiphenomena, and the primary pathogenesis the result of an immune response to an obscure antigen (eg, a virus).

The autoimmune reaction is probably normally held in check by the action of a population of specific suppressor T cells. Any of the above processes could lead to or be associated with a suppressor T-cell defect. A perturbation in the regulation of antibody activity by anti-idiotype antibodies (antibodies to the antigen-combining site of other antibodies) may play a role.

The roles of other complex mechanisms demonstrable experimentally still need clarification. For example, nonantigenic adjuvants (eg, alum, bacterial endotoxin) enhance the antigenicity of other substances. Freund's complete adjuvant, an emulsion of antigen in mineral oil with heat-killed mycobacteria, is usually required to produce autoimmunity in experimental animals.

Genetic factors play a role. Relatives of patients with autoimmune disorders often show a high incidence of the same type of autoantibodies, and the incidence of autoimmune diseas is higher in identical than fraternal twins. Women are affected more often than men. The genetic contribution appears to be one of predisposition. In a predisposed population, a number of environmental factors could provoke disease; eg, in SLE these might be latent virus infection, drugs, or tissue injury such as occurs with ultraviolet light exposure. This situation would be analogous to the development of hemolytic anemia as a consequence of

environmental factors in persons with G6PD deficiency (see Ch. 127), a predisposing genetically determined biochemical abnormality.

Pathogenesis

The pathogenetic mechanisms of autoimmune reactions are, in many cases, better understood than the way in which autoantibodies develop. In some autoimmune hemolytic anemias, the RBCs become coated with cytotoxic (type II) autoantibody; the complement system responds to these antibody-coated cells just as it does to similarly coated foreign particles, and the interaction of complement with the antibody complexed to the cell surfac antigen leads to RBC phagocytosis or cytolysis.

Autoimmune renal injury can occur as the result of either an antibody-mediated (type II) or IC (type III) reaction. The antibody-mediated reaction occurs in Goodpasture's syndrome, in which lung and renal disease is associated with an anti-basement membrane antibody (see Ch. 77). The best known example of autoimmune injury associated with soluble antigen-antibody complexes (ICs) is the nephritis associated with SLE (see Systemic Lupus Erythematosus in Ch. 50 and below). Another example is a form of membranous glomerulonephritis that is associated with an IC containing renal tubular antigen. Although not proven, poststreptococcal glomerulonephritis could be due in part to streptococcus-induced cross-reacting antibodies.

Various autoantibodies are produced in SLE and other systemic (as opposed to organ-specific) autoimmune diseases. Antibodies to formed elements in the blood account for autoimmune hemolytic anemia (see Ch. 127), thrombocytopenia, and possibly leukopenia; anticoagulant antibodies may cause disordered coagulation problems. Antibodies to nuclea material result in deposition of ICs, not only in glomeruli but also in vascular tissues and ir skin at the dermal-epidermal junction. Synovial deposition of aggregated IgG-rheumatoid factor-complement complexes occurs in RA. Rheumatoid factor is usually an IgM (occasionally IgG or IgA) with specificity for a receptor on the constant region of the heav chain of autologous IgG. The IgG-rheumatoid factor-complement aggregates can also be found within neutrophils, where they cause the release of lysosomal enzymes that contribute to the inflammatory joint reaction. Many plasma cells are present within the join and may synthesize anti-IgG antibodies. T cells and lymphokines are also found in rheumatoid joints and may contribute to the inflammatory process. The process that sets of the immunologic events is unknown; it could be a bacterial or viral infection. In SLE, the low serum complement level reflects the widespread immunologic reactions taking place; in RA, by contrast, serum complement is normal but intrasynovial complement levels are low.

In **pernicious anemia,** autoantibodies capable of neutralizing intrinsic factor are found in the GI lumen. Autoantibodies against the microsomal fraction of gastric mucosal cells are even more common. It is postulated that a cell-mediated autoimmune attack against the parietal cells results in the atrophic gastritis, which, in turn, reduces the production of intrinsic factor but still allows absorption of sufficient vitamin B_{12} to prevent the megaloblastic anemia. If autoantibodies to intrinsic factor should also develop in the GI lumen, however, B_{12} absorption would cease and pernicious anemia would develop.

Hashimoto's thyroiditis is associated with autoantibodies to thyroglobulin, the microsomes of thyroid epithelial cells, a thyroid cell surface antigen, and a second colloid

antigen. Tissue injury and eventual myxedema may be mediated both by the cytotoxicity of the microsomal antibody and by the activity of specifically committed T cells. Low-titered antibodies are also found in patients with primary myxedema, suggesting that it is the end result of unrecognized autoimmune thyroiditis. An autoimmune reaction is also involved in thyrotoxicosis **(Graves' disease),** and about 10% of patients eventually develop myxedema spontaneously; many more do so after ablative therapy. Other antibodies unique to Graves' disease are called thyroid-stimulating antibodies. They react with thyroid-stimulating hormone (TSH) receptors in the gland and have the same effect as TSH on thyroid cell function.